

WHAT IS CLAIMED IS:

1. A universal bystander cell line, which:
 - (i) is a human cell line,
 - (ii) naturally lacks major histocompatibility class I (MHC-I) antigens and
5 major histocompatibility class II (MHC-II) antigens or is modified so that it lacks
MHC-I antigens and MHC-II antigens, and
 - (iii) is modified by introduction of a nucleic acid molecule comprising a
nucleic acid sequence encoding granulocyte macrophage-colony stimulating factor
(GM-CSF) operably linked to a promoter,
10 wherein said universal bystander cell line expresses at least about 500 ng
GM-CSF/ 10^6 cells/24 hours.
2. The universal bystander cell line of claim 1, wherein said human cell
line is characterized by the absence of B-lymphocyte markers of immunoglobulin,
15 an Epstein-Barr virus (EBV) genome and an associated nuclear antigen, and
receptors for EBV.
3. The universal bystander cell line of claim 1, wherein said human cell
line is derived from a blast crisis of chronic myeloid leukemia.
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4. The universal bystander cell line of claim 1, wherein said human cell
line is K562.
5. The universal bystander cell line of claim 1, which expresses at least
25 about 1,000 ng GM-CSF/ 10^6 cells/24 hours.
6. The universal bystander cell line of claim 1, which grows in defined
medium.
- 30 7. The universal bystander cell line of claim 1, wherein said promoter is a
cytomegalovirus promoter.

8. The universal bystander cell line of claim 4, which expresses at least about 1,000 ng GM-CSF/ 10^6 cells/24 hours.

5 9. The universal bystander cell line of claim 4, which grows in defined medium.

10 10. The universal bystander cell line of claim 4, wherein said promoter is a cytomegalovirus promoter.

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11. The universal bystander cell line of claim 1, wherein said nucleic acid molecule further comprises a nucleic acid sequence encoding hygromycin resistance operably linked to a promoter and said universal bystander cell line is selected by growth in a culture medium comprising at least about 400 μ g/ml
15 hygromycin.

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12. The universal bystander cell line of claim 11, wherein said universal bystander cell line is selected by growth in a culture medium comprising at least about 1,000 μ g/ml hygromycin.

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13. The universal bystander cell line of claim 4, wherein said nucleic acid molecule further comprises a nucleic acid sequence encoding hygromycin resistance operably linked to a promoter and said universal bystander cell line is selected by growth in a culture medium comprising at least about 400 μ g/ml
25 hygromycin.

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14. The universal bystander cell line of claim 13, wherein said universal bystander cell line is selected by growth in a culture medium comprising at least about 1,000 μ g/ml hygromycin.

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15. A composition comprising:

(a) a universal bystander cell line, which

(i) is a human cell line, (ii) naturally lacks MHC-I antigens and MHC-II antigens or is modified so that it lacks MHC-I antigens and MHC-II antigens, and

5 (iii) is modified by introduction of a nucleic acid molecule comprising a nucleic acid sequence encoding an immunomodulatory cytokine operably linked to a promoter, and

(b) a cancer antigen.

10 16. The composition of claim 15, wherein said immunomodulatory cytokine is interleukin-2 (IL-2).

17. A composition comprising the universal bystander cell line of claim 1 and a cancer antigen.

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18. A composition comprising the universal bystander cell line of claim 2 and a cancer antigen.

19. A composition comprising the universal bystander cell line of claim 4 and a cancer antigen.

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20. A composition comprising the universal bystander cell line of claim 5 and a cancer antigen.

21. A composition comprising the universal bystander cell line of claim 8 and a cancer antigen.

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22. A method of making a universal GM-CSF-expressing bystander cell line, which method comprises:

30 (i) obtaining a human cell line that lacks MHC-I antigens and MHC-II antigens;

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(ii) modifying said human cell line by introducing into said human cell line a nucleic acid molecule comprising a nucleic acid sequence encoding GM-CSF operably linked to a promoter and a nucleic acid sequence encoding a selectable marker operably linked to a promoter; and

5 (iii) using the selectable marker to isolate cells that produce at least about 500 ng of said GM-CSF/ 10^6 cells/24 hours.

23. The method of claim 22, wherein said selectable marker is hygromycin resistance.

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24. The method of claim 23, wherein the modified human cell line is cultured in culture medium comprising at least about 400 μ g hygromycin/ml culture medium.

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25. The method of claim 24, wherein the modified human cell line is subsequently cultured in culture medium comprising at least about 1,000 μ g hygromycin/ml culture medium.

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26. The method of claim 24, wherein said culture medium is defined.

27. The method of claim 25, wherein said culture medium is defined.

28. The method of claim 22, wherein the promoter to which the nucleic acid sequence encoding GM-CSF is operably linked is a cytomegalovirus
25 promoter.

29. A method of making a universal GM-CSF-expressing bystander cell line, which method comprises:

(i) obtaining a human cell line;
30 (ii) modifying said human cell line so that it lacks MHC-I antigens and MHC-II antigens;

(iii) further modifying said human cell line by introducing into said human cell line a nucleic acid molecule comprising a nucleic acid sequence encoding GM-CSF operably linked to a promoter and a nucleic acid sequence encoding a selectable marker operably linked to a promoter; and

5 (iv) using the selectable marker to isolate cells that produce at least about 500 ng of GM-CSF/ 10^6 cells/24 hours.

30. The method of claim 29, wherein said selectable marker is hygromycin resistance.

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31. The method of claim 30, wherein the modified human cell line is cultured in culture medium comprising at least about 400 μ g hygromycin/ml culture medium.

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32. The method of claim 31, wherein the modified human cell line is subsequently cultured in culture medium comprising at least about 1,000 μ g hygromycin/ml culture medium.

33. The method of claim 31, wherein said culture medium is defined.

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34. The method of claim 32, wherein said culture medium is defined.

35. The method of claim 29, wherein the promoter to which the nucleic acid sequence encoding GM-CSF is operably linked is a cytomegalovirus promoter.

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36. A method of stimulating an immune response to a cancer in a human patient, which method comprises administering to said patient the composition of claim 15, wherein said cancer antigen is an antigen of said cancer and wherein said composition is irradiated,

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whereupon administration of said composition, an immune response to said cancer is stimulated.

37. The method of claim 36, wherein said cancer antigen is a cell of said
5 cancer.

38. A method of stimulating an immune response to a cancer in a human patient, which method comprises administering to said patient the composition of claim 16, wherein said cancer antigen is an antigen of said cancer and wherein said
10 composition is irradiated,

whereupon administration of said composition, an immune response to said cancer is stimulated.

39. The method of claim 38, wherein said cancer antigen is a cell of said
15 cancer.

40. A method of stimulating an immune response to a cancer in a human patient, which method comprises administering to said patient the composition of claim 17, wherein said cancer antigen is an antigen of said cancer and wherein said
20 composition is irradiated,

whereupon administration of said composition, an immune response to said cancer is stimulated.

41. The method of claim 40, wherein said cancer antigen is a cell of said
25 cancer.

42. A method of stimulating an immune response to a cancer in a human patient, which method comprises administering to said patient the composition of claim 19, wherein said cancer antigen is an antigen of said cancer and wherein said
30 composition is irradiated,

whereupon administration of said composition, an immune response to said cancer is stimulated.

43. The method of claim 42, wherein said cancer antigen is a cell of said
5 cancer.

44. A method of stimulating an immune response to a cancer in a human patient, which method comprises administering to said patient the composition of claim 20, wherein said cancer antigen is an antigen of said cancer and wherein said
10 composition is irradiated,

whereupon administration of said composition, an immune response to said cancer is stimulated.

45. The method of claim 44, wherein said cancer antigen is a cell of said
15 cancer.

46. A method of stimulating an immune response to a cancer in a human patient, which method comprises administering to said patient the composition of claim 21, wherein said cancer antigen is an antigen of said cancer and wherein said
20 composition is irradiated,

whereupon administration of said composition, an immune response to said cancer is stimulated.

47. The method of claim 46, wherein said cancer antigen is a cell of said
25 cancer.

48. In a method of cancer immunotherapy, the improvement comprising administering to a human patient having a cancer the composition of claim 15, wherein said cancer antigen is an antigen of said cancer and wherein said
30 composition is irradiated.

49. In a method of cancer immunotherapy, the improvement comprising administering to a human patient having a cancer the composition of claim 16, wherein said cancer antigen is an antigen of said cancer and wherein said
5 composition is irradiated.

50. In a method of cancer immunotherapy, the improvement comprising administering to a human patient having a cancer the composition of claim 17, wherein said cancer antigen is an antigen of said cancer and wherein said
10 composition is irradiated.

51. In a method of cancer immunotherapy, the improvement comprising administering to a human patient having a cancer the composition of claim 19, wherein said cancer antigen is an antigen of said cancer and wherein said
15 composition is irradiated.

52. In a method of cancer immunotherapy, the improvement comprising administering to a human patient having a cancer the composition of claim 20, wherein said cancer antigen is an antigen of said cancer and wherein said
20 composition is irradiated.

53. In a method of cancer immunotherapy, the improvement comprising administering to a human patient having a cancer the composition of claim 21, wherein said cancer antigen is an antigen of said cancer and wherein said
25 composition is irradiated.

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